

Exploring the diversity of methane-oxidizing bacteria in marine ecosystems

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Methane-oxidizing bacteria (MOB), or methanotrophs, are an important subset of a physiological group of bacteria known as methylotrophs. Methanotrophic bacteria are unique in their ability to utilize methane as their sole carbon and energy source (Hanson and Hanson, 1996). On a yearly scale they consume on average 30 Tg of atmospheric CH₄ (range: 15–45 Tg y⁻¹; 6% of the global sink). Furthermore they exhibit a wide range of growing conditions and they contain key enzymes with a remarkable broad substrate specificity (Kolb, 2009; Semrau *et al.*, 2010). Thanks to their versatile nature they exhibit a high potential for application in industrial biotechnology. So far the focus of most isolation studies has been the terrestrial ecosystems, because of their large annual methane emissions. However marine ecosystems, which annually contribute for only 2% of the total methane emission, have been ignored. As a result little information is available about MOB diversity in these ecosystems, with only four species named and described so far (Hirayama *et al.*, 2012).

To this end we investigated the cultivable MOB diversity in sediment samples of six North Sea stations positioned from near shore to open ocean along an increasing salinity and decreasing anthropogenic nitrogen gradient respectively. For each station enrichments were performed at different oxygen penetration depths. In total 24 enrichments were performed under *in situ* nutrient concentrations. Subsequently each enrichment was subjected to a high-throughput miniaturized dilution-to-extinction series under different nitrogen and oxygen concentrations, resulting in a total of 288 enrichments of which 206 scored positive for MOB activity. All positive cultures were thereafter transferred to gellum gum plate to isolate the MOB. From our data it appears that there is a decreasing trend of cultivability of MOB on solid plates from near shore stations towards more open sea stations. Furthermore all isolates obtained so far resist purification, even after extensive subcultivation and extra dilution-to-extinction series, indicating microbial interactions plays a crucial role in the survival of these marine MOB. A further identification via a molecular *pmoA* based approach only resulted in the identification of half of the cultures.

Currently we are trying to identify the active MOB in the unknown cultures by following a two-step strategy with (i) Ion torrent shot gun sequencing of 4 cultures and (ii) by pursuing alternative isolation strategies (e.g. floating filter isolation) to isolate and subsequently identify the MOB.